PCT/EP00/10058
Connex Gesellschaft zur Optimierung ...
u. Z.: D 2394 PCT

Amended Claims

1. A method for detecting an infection of a mammal with an acid-resistant bacterium belonging to the genus Helicobacter or Campylobacter, wherein

   (a) a stool sample of the mammal is incubated with (aa) a receptor under conditions permitting a complex formation of an antigen from the acid resistant bacterium with the receptor; or (ab) two different receptors under conditions permitting a complex formation of an antigen from the acid-resistant bacterium with the two receptors and wherein the receptor according to (aa) or the receptors according to (ab) specifically bind(s) an antigen which shows, at least with some mammals, a structure after passage through the intestine that corresponds to the native structure or the structure which a mammal produces antibodies against after being infected or immunized with the acid-resistant bacterium or an extract or lysate thereof or a protein therefrom or a fragment thereof or a synthetic peptide; and

   (b) wherein the formation of at least one antigen-receptor complex according to (a) is detected.

2. The method according to claim 1, wherein the bacterium is a bacterium belonging to the species *Helicobacter pylori, Helicobacter hepaticus* or *Campylobacter jejuni.*

3. The method according to claim 2 or 3, wherein the antigen is the antigen of a catalase, a urease or a metalloproteinase.

4. The method according to claim 3, wherein the antigen is an antigen of H. pylori.

5. The method according to any one of claims 1 to 4, wherein the receptor/the receptors is (are) (an) antibody(ies), (a) fragment(s) or derivative(s) thereof or (an) aptamer(s).

6. The method according to any one of claims 1 to 5, wherein for the detection additionally a mixture of receptors is used, wherein the mixture of receptors has the function of catching the antigen if the receptor is used as detector of the antigen, and the mixture has the function of detecting the antigen if the receptor is used as catcher of the antigen.

7. The method of claim 6, wherein the mixture of receptors is a polyclonal antiserum.

8. The method according to claim 7, wherein the polyclonal antiserum is obtained against a lysate of the bacterium.

9. The method according to claim 8, wherein the lysate is a lysate with enriched antigen.

10. The method according to claim 8 or 9, wherein the lysate is a lysate with depleted immunodominant antigens.

11. The method according to claim 7, wherein the polyclonal antiserum is obtained against a purified or a (semi-)synthetically produced antigen.

12. The method according to any one of claims 1 to 11, wherein the antigen is an antigen of a catalase, a urease or a metalloproteinase.

13. The method according to any one of claims 1 to 12, wherein the receptor and/or the mixture of receptors bind(s) (a) conformation epitope(s).

14. The method according to any one of claims 5 to 13, wherein the heavy chain of the antibody binding a catalase epitope has at least one of the following CDRs, preferably the CDR3:

CDR1:	NYWIH
CDR2:	YINPATGSTSYNQDFQD
CDR3:	EGYDGFDS

15. The method according to claim 14, wherein the heavy chain has all the three CDRs mentioned.

16. The method according to claim 14 or 15, wherein the DNA sequence encoding the heavy chain of the antibody has at least one of the following CDRs, preferably the CDR3 and still more preferably all of the three following CDRs:

CDR1: AACTACTGGA TTCAC

CDR2: TACATTAATC CTGCCACTGG TTCCACTTCT TACAATCAGG ACTTTCAGGA C

CDR3: GAGGGGTACG ACGGGTTTGA CTCC

17. The method according to any one of claims 5 to 13, wherein the light chain of the antibody binding a catalase epitope has at least one of the following CDRs, preferably the CDR3 and still more preferably all of the three following CDRs:

CDR1: SASSSVNYMY

CDR2: DTSKLAS

CDR3 . QQWSSNPYT

18. The method according to claim 17, wherein the DNA sequence encoding the light chain of the antibody has at least one of the following CDRs, preferably the CDR3 and still more preferably all of the three following CDRs:

CDR1: AGTGCCAGCT CAAGTGTAAA TTACATGTAC

CDR2: GACACATCCA AATTGGCTTC T

CDR3: CAGCAGTGGA GTAGTAATCC GTACACG

19. The method according to any one of claims 5 to 13, wherein the heavy chain of the antibody binding a catalase epitope exhibits at least one of the following CDRs, preferably the CDR3 and more preferably all three of the following CDRs:

CDR1: DTYVH

CDR2: KIDPANGKTKYDPIFQA

CDR3: PIYYASSWFAY

20. The method according to claim 19, wherein the DNA sequence encoding the heavy chain of the antibody exhibits at least one of the following CDRs, preferably CDR3 and more preferably all three of the following CDRs:

CDR1: GACACCTATGTGCAC

CDR2: AAGATTGATCCTGCGAATGGTAAAACTAAATATGACCC GATATTCCAGGCC

CDR3: CCCATTTATTACGCTAGTTCCTGGTTTGCTTAC

21. The method according to any one of claims 5 to 13, wherein the light chain of the antibody binding a catalase epitope exhibits at least one of the following CDRs, preferably CDR3 and more preferably all three of the following CDRs:

CDR1: KASQDVGTSVA

CDR2: WTSTRHT

CDR3: QYSSSPT

22. The method according to claim 21, wherein the DNA sequence encoding the light chain of the antibody exhibits at least one of the following CDRs, preferably CDR3 and more preferably all three of the following CDRs:

CDR1: AAGGCCAGTCAGGATGTGGGTACTTCTGTTGCC

CDR2: TGGACATCCACCCGGCACACT

CDR3: CAGCAATATAGCAGCTCTCCCACG

23. The method according to any one of claims 5 to 13, wherein the heavy chain of the antibody binding an epitope of the β-urease exhibits at least one of the of the following CDRs, preferably CDR3 and more preferably all of the three following CDRs:

CDR1: GFTFSSHFMS

CDR2: SISSGGDSFYPDSLKG

CDR3: DYSWYALDY

or:

CDR1: GYAFSTSWMN

CDR2: RIYPGDGDTNYNGKFKG

CDR3: EDAYYSNPYSLDY

24. The method according to claim 23, wherein the DNA sequence of the antibody encoding the heavy chain exhibits at least one of the of the following CDRs, preferably CDR3 and more preferably all three of the following CDRs:

CDR1: GG CTACGCATTC AGTACCTCCT GGATGAAC

CDR2: CGGATTTATC CTGGAGATGG AGATACTAAC TACAATGGGA AGTTCAAGGG C

CDR3: GAG GATGCCTATT ATAGTAACCC CTATAGTTTG GACTAC

or:

CDR1: GG ATTCACTTTC AGTAGCCATT TCATGTCT

CDR2: TCCATTAGTA GTGGTGGTGA CAGTTTCTAT CCAGACAGTC TGAAGGGC

CDR3: GACTAC TCTTGGTATG CTTTGGACTA C

25. The method according to any one of claims 5 to 13, wherein the light chain of the antibody binding an epitope of the β-urease exhibits at least one of the of the following CDRs, preferably CDR3 and more preferably all three of the following CDRs:

CDR1: RASQSIGTRIH

CDR2: YGSESIS

CDR3: QQSNTWPLT

or:

CDR1: HASQNINVWLS

CDR2: KASNLHT

CDR3: QQGRSYPLT

26. The method according to claim 25, wherein the DNA sequence encoding the light chain of the antibody exhibits at least one of the of the following CDRs, preferably CDR3 and more preferably all three of the following CDRs:

CDR1: A GGGCCAGTCA GAGCATTGGC ACAAGAATAC AC
CDR2: TAT GGTTCTGAGT CTATCTCT
CDR3: CAACAA AGTAATACCT GGCCGCTCAC G
or:
CDR1: C ATGCCAGTCA GAACATTAAT GTTTGGTTAA GC
CDR2: AAG GCTTCCAACT TGCACACA
CDR3: CAACAG GGTCGAAGTT ATCCTCTCAC G

27. The method according to any one of claims 5 to 26, wherein the antibodies in the variable regions of the light and heavy chains have the amino acid sequences shown in Figures 1 and 2, 3 and 4, 5 and 6 or 7 and 8.

28. The method according to any one of claims 5 to 27, wherein the coding regions of the variable regions of the light and heavy chains have the DNA sequences shown in Figures 1 and 2, 3 and 4, 5 and 6 or 7 and 8.

29. The method according to any one of claims 1 to 28, wherein the following steps are carried out with the stool sample before incubation with the antibodies:
    (a) resuspending the stool sample at a ratio of 1:3 to 1:25, preferably approximately at a ratio of 1:5 to 1:10, particularly preferably 1:5, in resuspension buffer and
    (b) mixing on a vortex mixer.

30. The method according to any one of claims 1 to 29, wherein the detection of the formation of the at least one antigen-receptor complex/antigen-receptor receptor-mixture complex in step (b) takes place by means of an immunological method.

31. The method according to any one of claims 1 to 30, wherein the detection of the formation of the at least one antigen-receptor complex/antigen-receptor/receptor-mixture complex in step (b) takes place by means of ELISA, RIA, Western blot or an immunochromatographic method.

32. The method according to claim 30 or 31, wherein in RIA or in ELISA the same receptor is used for both binding to the solid phase and detecting the epitope.

33. The method according to any one of claims 1 to 32, wherein the receptor is fixed to a support.

34. The method according to any one of claims 1 to 33, wherein the receptor is a monoclonal murine antibody.

35. The method according to any one of claims 1 to 34, wherein the method is a one-step ELISA.

36. The method according to any one of claims 1 to 34, wherein the method is a three-step ELISA.

37. The method according to claim 33, wherein the material of the support is a porous material.

38. The method according to claim 33 and 37, wherein the material of the support is a test strip.

39. The method according to claims 33, 37 or 38, wherein the material of the support consists of cellulose or a derivative of cellulose.

40. The method according to any one of claims 1 to 39, wherein breath condensate, gastric gas, tooth plaque, saliva, mucous smear, biopsy, whole blood or serum is used for the detection instead of a stool sample.

41. The method according to any one of claims 1 to 40, wherein the method is a automated method.

42. The method according to any one of claims 1 to 41, wherein the mammal is a human.

43. A monoclonal antibody, fragment or derivative thereof which has a V region that shows a combination of the CDRs illustrated in any one of claims 14 to 26.

44. The monoclonal antibody, fragment or derivative thereof according to claim 43 which has at least one of the V regions shown in Figures 1 and 2, 3 and 4, 5 and 6 or 7 and 8.

45. The monoclonal antibody, fragment or derivative thereof according to claims 43 and 44 which is a murine antibody or a fragment or derivative thereof or a chimeric, preferably a humanized antibody or a fragment or derivative thereof.

46. An aptamer which specifically binds the same epitope as the monoclonal antibody, the fragment or derivative thereof according to any one of claims 43 to 45.

47. An epitope which is specifically bound by the monoclonal antibody, fragment or derivative thereof according to any one of claim 43 to 45 or the aptamer according to claim 46.

48. The antibody, fragment or derivative thereof which specifically binds the epitope according to claim 47.

49. Diagnostic composition containing at least one receptor as defined in any one of the aforementioned claims, optionally fixed to a support material, wherein said diagnostic composition optionally further contains a mixture of receptors as defined in any one of the aforementioned claims, optionally fixed to a support material.

50. A test device for the detection of at least one of the epitopes as defined in any one of the aforementioned claims comprising

    (a) at least one receptor as defined in any one of the aforementioned claims fixed to a support material;
    (b) a device for preparing and analysing stool samples; and optionally
    (c) a mixture of receptors as defined in any one of the aforementioned claims.

51. A test device for the detection of at least one epitope as defined in any one of the aforementioned claims comprising

    (a) at least one receptor as defined in any one of the aforementioned claims, wherein the receptor is conjugated with colloidal gold, latex particles or other colouring particles the size of which typically ranges between 5 nm and 100 nm, preferably between 20 nm and 60 nm, particularly preferably between 40 nm and 60 nm (gold) and 200 nm and 500 nm (latex);
    (b) a device for preparing and analysing stool samples; and optionally
    (c) a mixture of receptors as defined in any one of the aforementioned claims.

52. A kit containing

(a) at least one receptor as defined in any one of the aforementioned claims, optionally fixed to a support; optionally furthermore
(b) a device for preparing and analysing stool samples; and optionally
(c) a mixture of receptors as defined in any one of the aforementioned claims.

53. A composition, preferably a pharmaceutical preparation containing at least one of the above-described receptors, optionally in combination with a pharmaceutically acceptable support and/or diluent.

54. A package containing the diagnostic composition according to claim 49, the test device according to claims 50, 51 or the kit according to claim 52.